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Genome-wide association studies on growth performance trait of Nigerian indigenous Chicken genotypes

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Abstract

Meat from Nigerian indigenous chickens (NICs) has high consumer demands but slow growth rate with high variability in body weight (BW). A study was carried out at T&R farm of FUNAAB identifying chromosomes associated with body weights of NICs in a GWAS study using the chicken 60K Single Nucleotide Polymorphisms (SNPs) Panel for 53313 markers subjected to test. Body weight was taken for 8 week. Blood samples were collected from 42 purebreds and F_2 upgrades of NICs to extract DNA and amplify through PCR-RFLP technique to obtain Amplicons used for sequencing. Sequenced molecular analysis involving many Molecular software packages and bioinformatics approach were were done to trim and compare the sequence. The raw phenotypic data which comprised of the sex and body weight (>2 kg as 1 (case)) and <1 kg as 0 (control)) were analyzed using Gen Abel of the R Software Package to carry out quality checks through some filtering techniques on these chickens. The results from Selective sweep analysis identified ten chromosomes and SNPs associated with BWT of the NICs F_2 upgrades Nigerian Chickens resource population. However, ten chromosomes with SNPs associated with BWT at the suggestive Bonferroni significant threshold level ($p < 0.05$) were discovered. These findings made it clear that F2 upgrades had better trait association on chromosomes when compared to those of the Purebreds and should be included as a genetic marker in the breeding programs by breeders for genetic improvement due to the potentialities found in this NICs genotypes studied.

Keywords: Bonferroni Significance threshold; Chromosomes; Genome-Wide Association; Growth trait; Indigenous Chickens; Single Nucleotide Polymorphisms.

1. Introduction

Knowledge of genes contributing to chicken growth can be used to identify polymorphisms of these genes in production lines to improve breeding program efficiency. The means to identify these genes related to complex traits at the genome-wide level emerged from 1990s through the approach of Mapping of quantitative trait loci (QTL) to detect genetic variation for economically important traits as body weight at the genome level to date. Thousands of QTLs for numerous traits have been reported (http://www.animalgenome.org/QTL/db) following this approach. Previously, microsatellite markers with low map resolution and the confidence interval (CI) were used in detecting the QTLs which covers over 20 cM or a whole chromosome but the problem of identification of causal mutations underlying QTLs and detection of gene associated with each trait of interest in domestic animals based on the information obtained. Therefore, the genome wide association study (GWAS) which is one of the new techniques for the identification of causal genes for important traits in livestock is quite ideal to harness the challenges faced in the identification technique. The use of GWAS in chickens has been to ascertain major loci related to economic traits [1; 2; 3]. Most economic traits in animals display quantitative variation, which is regulated by many QTLs with comparatively small effects and altered by the environment [4]. Growth performance traits are among the furthermost important economic traits in a poultry venture [5]. Significant improvements in the study of growth performance traits in chicken have been realized, and many related

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genes and QTLs have been reported [3]. Over 1,500 QTLs, covering the majority of the chicken genome has been linked with growth performance traits [6; 7; 8]. The majority of these reported QTLs are from crossbred chicken populations (Mebratie *et al*. [9] and were discovered using microsatellites, markers with low map resolution, resulting in the detection of a few causative genes [7].

A GWAS in chicken body weight by Gu *et al*. [10] was observed that a total of 26 SNP effects related to 9 different SNPs were significantly associated with body weight at 7–12 weeks of age. These significant SNPs were mainly in a region of the chicken chromosome 4 approximately 8.6 Mb in length (71.6–80.2 Mb). The LIM domain-binding factor 2 (LDB2) gene in this region had the strongest association with body weight for weeks 7–12, and with average daily gain for weeks 6–12. This gene may be important in the regulation of body weight in the chicken.

Another GWAS on chicken growth was reported by Xie *et al. [11]* which observed that a total of 257 SNP effects involving 68 SNPs and 23 genes were detected for 18 traits with genome-wide significant. Among these identified SNPs or regions, the 1.5 Mb region (173.5–175 Mb) of chicken chromosome (GGA) 1 was the most important for chicken growth traits and genes in this region may be important for chicken growth. However, this study focused on the association studies on the growth performance in Nigerian indigenous Chicken genotypes.

2. Material and methods

2.1. Experimental site

The research was carried out at the Poultry Breeding Unit of the University Teaching and Research Farm of Federal University of Agriculture, Abeokuta, Alabata Road, Abeokuta. Alabata is at latitude 7°10`N and longitude 3°2`E in Odeda Local Government Area of Ogun State, Nigeria. The area is characterized with a tropical climate with a mean annual rainfall of about 1037mm. The mean monthly ambient temperature ranged from 28- 36°C and 34°C on average. Relative humidity ranged from 60-94%, 82% on the average. The vegetation represents an inter-phase between the tropical rainforest and the derived savannah [12].

2.2. Experimental Management

2.2.1. Parents:

Pure indigenous chicken genotypes reared in the breeding unit of the University's Research and Teaching Farm were used to generate the offspring for this research. The parent stock were basically Normal-feathered (N) of two cocks and ten hens, Frizzled-Feathered (Fz) of one Cock and seven hens and naked neck chicken (NK) of two 2 Cocks and ten hens and Marshall (M) of three Cocks and hens and ten di-hybrid cross of ten hens which were cross through artificial insemination method. The combination of these four genotypes at different bloodlines resulted in ten genotypes used in this study: 100% pure breeds, 50:50% M: Ind (di-hybrid) 75:25% M: Ind (F_2 upgrade) fifty chickens per genotype (sample size) making five hundred chickens altogether was the population for the chickens. The genotypes are presented below:

2.2.2. Mating design

- M X M = M
- N X N = N
- Fz X Fz = Fz
- Nk X Nk = Nk

50%:50% (M: Ind) bloodline (F_1crosses)

- M X N = MN (F_1)
- M X Fz = MFz (F_1)
- M X Nk =MNk (F_1)

75%:25% (M: Ind) bloodline (F_2 upgrades)

- M X MN =MMN (F_2)
- M X MF =MMFz (F_2)
- M X MNk =MMNk (F_2)

M- Marshall Exotic breed, N-Normal-Feathered, Fz-Frizzle-Feathered,

Nk-Naked Neck chicken genotype, M: Ind = Marshall: indigenous.

2.2.3. Inseminations, egg collection and hatching:

Artificial insemination was carried out on the chickens three times a week. It was done by introducing semen collected from Exotic Marshall cocks (sires) into the reproductive tracts of both exotic and indigenous hens (dams), also, indigenous cock to Indigenous hens following the above mating design to produce the 10 different genotypes of birds studied in this research. The parent stock was housed in the battery cage system singly and fed *ad libitum* with constant water supplied. The pure Marshall genotype served as control. Then the same exotic cocks (Marshal) were crossed to all the genotypes to obtain F_1 birds with 50%: 50% M: Ind bloodline (dihybrid crosses) and F_2 birds with 75%:25% M: Ind bloodline (F_2 upgrades).

2.2.4. Egg collection, incubation and hatching:

Eggs from each mating group were collected daily and labelled according to sire-line. The eggs were then transferred to the University's Teaching and Research farm hatchery unit for incubation and hatching. Assortment of eggs for incubation was done by selecting cracked, discolored or odd- shaped eggs before setting. The eggs were fumigated so as to destroy microbes using fumes from mixture of 35g potassium permanganate in 53cc formalin per cubic meters. Circular air movement in the cabinet and speedy gas removal after 20 minutes fumigation was ascertained. Three day egg collections were set along sire-lines at a temperature of 38-39°C and humidity of 55-66% for the first eighteen days. On the 18th day of incubation, candling was conducted and number of fertile eggs were recorded. The eggs were transferred into the hatcher; the temperature was set to 39-40°C and humidity to 70-75%. The eggs were turned automatically hourly through 90°C in the incubator. On the 21st day chicks were collected and the number of hatched chicks was recorded.

2.3. Data collection:

2.3.1. Growth:

The chicks body weights (BW) were weighed weekly from day old to 8 weeks of age with the use of a weighing scale with 0.1g limit of sensitivity in the morning before feeding. Other linear body measurements were taken from day-old to 8 weeks using tape rule.

2.3.2. Statistical analyses:

Analysis of variance for the body parameters was carried out with [13] software using General Linear Model. Means were separated using Duncans Multiple Range Test at probability of 5% level.

Experimental models:

Model 1 :

- $Y_{ijk} = \mu + G_i + \varepsilon_{ij}$
- Y_{ijk} = Observation of the $_i^{th}$ genotype on the $_j^{th}$ birds
- μ = Overall mean
- G_i = Effect of the $_i^{th}$ chick genotype ($_i$ = 1, 2 …10)
- ε_{ij} = Error due to individual observation

2.4. Genomics

2.4.1. Blood collection

A total number of 200 chickens (offspring), 20 birds per a genotype at equal ratio of purebred and F_2 upgrade were randomly selected from the population of 500 chickens. 200 blood samples were collected from the wing vein of the chickens using needle and syringe and were transferred to Whatman FTA® Classic cards by dropping it within the printed circle area. The samples were air dried and then sealed in plastic bag containing silica gel beads. A selection based on representation of sex, genotypes on 8th week for body weight at the lower and upper quartiles of the groups were used to select the 42 samples used for genomic studies.

2.4.2. DNA extraction, purification and genotyping

Genomic DNA was extracted from the air-dried blood samples of 42 pure and improved Nigerian indigenous chickens at 8 weeks preserved on FTA cards following the FTA extraction method's protocol and the quality and concentration of genomic DNA was quantified using nanodrop® spectrophotometer and was diluted to 50 ng/µl to fulfil the requirements for the Illumina Infinium SNP genotyping platform. Genotyping using the Illumina 60 K Chicken SNP Bead chip assay was carried out at the Illumina-certified service provider, DNA Land Marks Inc., Canada according to the manufacturer's protocol. Quality control, test of population sub-structure using clutering algorithm and association analyses was performed in the R package v2.15.0 environment [14]. Combinations of function from the R base, locally developed scripts and GenABEL v1.7.2 package [15] ment core Teaf Developor R[16] was performed, using the check-marker function (attributes: maf=0.05, call= 0.95, p.lev=0ibs.mrk ="ALL", ibs, threshold = 0.95). Prunning was performed on the dataset prior to SNP association tests.

2.4.3. Statistical analysis on genome-wide association study (GWAS)

Association tests

The fast score test for association between a trait and genetic polymorphism were analyzed with GenABEL in R package using function qtscore to perform tests of allelic association of body weight at 8 weeks and genome-wide SNP polymorphism determined from the Illumina chip platform.

Test model formula:

qtscore (BWT~ sex,data=data, trait.type = "binomial", times = 1, quiet = FALSE, bcast = 10, clambda = TRUE, propPs = 1, details = TRUE)

Where body weight at week 8 (BWT) is a variable dependent on the covariate gen assuming the trait data set is binomial in nature.

The model BWT~sex (i.e.phenotypic data) used a general linear model (GLM) and later residuals when score test was computed for each of the SNPs in the analysis. Coefficients of regression were then reported for the body weight.

False discovery rate i.e. error involved (test of individual high heterozygosity) was corrected with P-value at 5% significant level [17] and overlapping sliding windows of 20 kb was used in selective sweeps using this formula: $Z^{(x+1)} = Z\ (y^x+1)\ /2)$.

x = the window index.

y = the number of SNPs in window index

Z = the position of SNP y.

The mean allele frequency (f) at the minor allele for SNPs within a window was calculated with this formula:

z $T^pf = (T^pf - \mu f)/\delta f$ and $T^Af = (T^Af - \mu f)/\ \delta f$

T^pf = the allele frequencies of samples exhibiting the trait of interest

T^Af = the allele frequencies of samples not exhibiting the trait of interest

μ = mean

δ = standard deviation of mean allele frequencies across the dataset.

Z = $({}_zT^pf - T^Af)$ final comparative score, indicating the number of deviations from the mean. An arbitrary threshold for significance was 5% using Bonferroni significance threshold.

The genomic region containing the most significant SNPs of a peak was explored by inspecting a 1Mb window around the location of this SNP using GioMart tool and the Ensemble genes 69 database to interrogate 500kb to each side of the marker using the UMD v 3.1 assemblies for the genomic region containing the most significant SNP.

The chicken QTLdb database was also examined to find out if the significant SNP mapped against any described chicken QTL (Quantitative trait loci).

3. Results and discussion

3.1. Genome-wide association analysis

A genome-wide association study using the chicken 60 k SNP panel in Nigerian chicken population derived from the cross between Marshall and pure Nigerian chickens. The raw phenotypic data which comprises of the sex, genotype and body weight (>2 kg as 1 (case)) and (<1 kg as 0 (control)) was analyzed using GenAbel of the R package software by applying the commands on the custom scripts to carry out quality check in the association test in Nigerian chickens and their crosses. A total of 42 Nigerian indigenous chickens (samples) at 8 weeks of age were used for the SNPs associated test with the body weight of this chicken population. 53313 markers were used for the SNP effects at 5 % Bonferroni genome-wide significance threshold level.

3.1.1. Quality checks using genotypic quality control:

To determine the association of SNPs with body weight of Nigerian chickens and their crosses with Marshall at 8 weeks of age genotype and phenotype were connected by converting phenotypic data to genotypic data and analyzed using GWAS method which involved 5 steps at 5 % to 95 % screening rate.

From the result of the quality check control in which body weight of Nigerian indigenous chickens have been subjected to proof if the Nigerian indigenous chickens had deviated Hardy Weinberg Equillibrum (HWE) principle through the Case/control analyses carried out in 42 Nigerian indigenous chicken samples at 8 weeks.

Filtering was done to exclude all false discovery rates such as too high heterozygosity, minor allele frequency, identity by state. These errors were reduced through check marker quality control approach which tends to have shifted the principles of HWE.

Call rate was the first criteria to screen the chicken samples: At high call rate of 95 %, total number of 53313 markers were subjected to each chicken sample in all the 42 chickens at 8 weeks of age involved in this filtering exercise. No chicken was removed at this call rate in the first step for not satisfying the test requirement and none was also removed for deviating HWE test.

Then the test with low 5 % minor allele frequency criteria was carried out and 5374 markers (10.08 %) were removed for not satisfying the requirement. Then low call rate of 95 % was adopted, 2293 markers (4.20 %) were removed for failing the requirement and no marker was removed due to deviating from HWE principle under this test.

Test for heterozygosity (FDR) was carried out and no marker was removed. Mean heterozygosity (FDR) was 0.26±0.09.

The chickens were checked with the criteria of too high identity by state (ibs) of 95 % using 46602 markers with 42 chickens. None was removed for not passing the tested condition. The mean ibs (identity-by -state) was 0.64±0.03. This was based on 46602 (87.41 %) autosomal markers. At the end of it all the 42 Nigerian indigenous chickens passed through all the tests. The second run with the same procedure was repeated but there was no change in the result from the first run.

The graphs represented both the case (BW>2 kg) and control (BW <1 kg) to show the test of HWE using Chi square formula approach. The graph was plotted as observed (x^2) body weight (phenotypic data) against expected (x^2) (HWE constant values) in Figures 1 and 2.

The graph in Figure 1 compared HWE expected data with the growth data (body weight) > 1 kg for purebreds Nigerian indigenous chicken at 8 weeks of age obtained from this study. The y-axis was the phenotypic data (body weights) known as observed data (x^2) against HWE constant values on the x-axis known as expected (x^2). From the graph it was derived that there was deviation from the standing principle of Hardy Weinberg Equilibrium as both values went at parallel direction with each other. The black line represented the HWE values while the thick large black line was the phenotypic values and the red line indicated the corrected phenotypic data after errors removal.

However, the lines were a little closer to each other, indicating that the slight to no deviation among the Nigerian purebred indigenous genotypes exhibiting lighter weights used in the control comparison. This little deviation could be due to migration.

Figure 2 for case (body weight) <2 kg comparison between the Nigerian F_2 upgrades genotypes with heavier body weight at 8 weeks of age and the graph was presented in the same pattern of the control. The phenotypic data (body weight) of Nigerian F_2 upgrades at 8 weeks of age was the observed data (x^2) against expected (x^2) which was the HWE values. From the graph it was derived that there was deviation from the standing principle of Hardy Weinberg Equilibrium as both values went at parallel direction with each other. The black line represented the HWE value while the thick large black line was the phenotypic values and the red line indicated after errors were corrected. Similarly, the lines were wide apart from each other, indicating deviation from HWE and this was experienced in the F_2 upgrades of the Nigerian Improved Indigenous breeds which were affected by the crossbreeding and selection. This confirmed that crossbreeding and selection could be responsible for the genetic variation (mutation). However, an analysis of global FST showed deviations from HWE as a result of inbreeding coefficient (FIS) and in total population was low to high. F_{IS} was 0.02 and F_{IT} was 0.99.

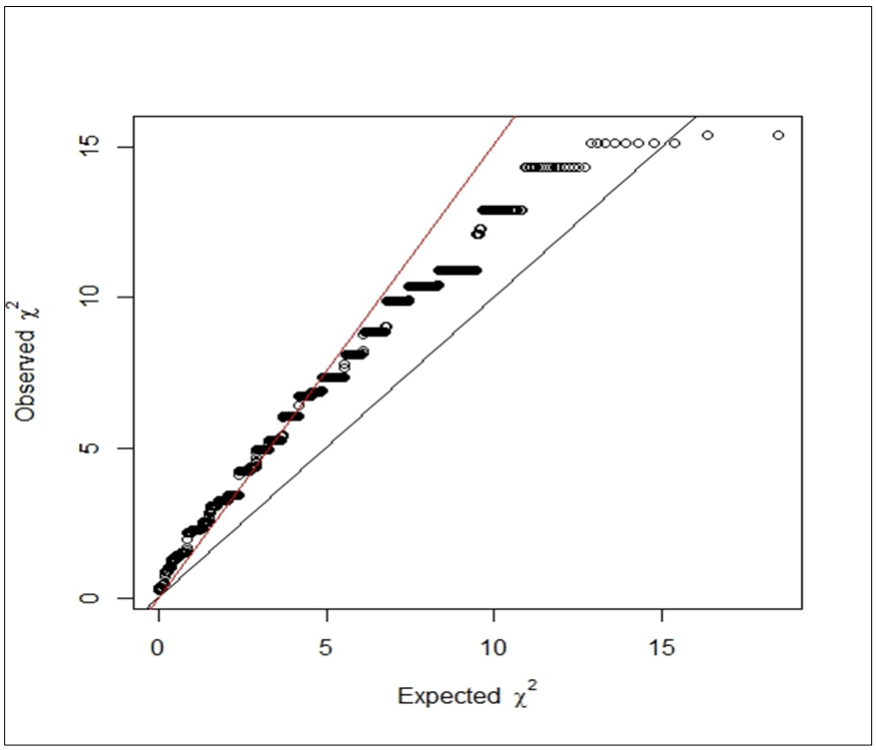
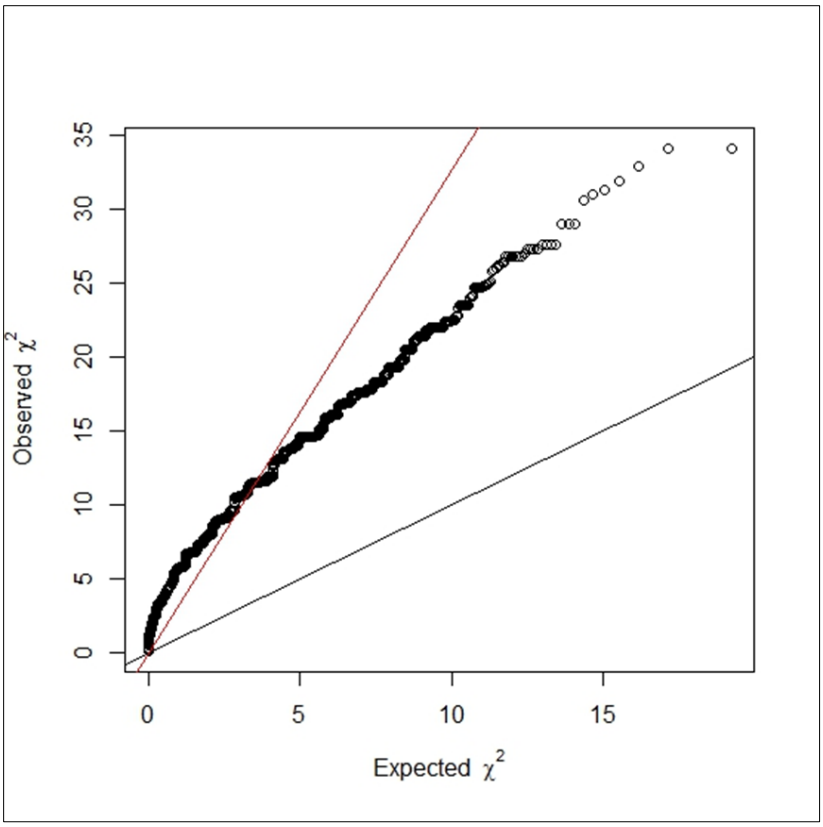


Figure 1 Graph of comparison between Hardy Weinberg Equillibrum principle and body weight of Nigerian indigenous chicken (control i.e >1 kg) at 8 weeks. (Thick black line –the observed body weight data i.e phenotypic data of Nigerian pure indigenous chickens at 8 week; Thin black line – the expected values of Hardy Weinberg Equilibrium; Red line – corrected values).



(Thick black line –the observed body weight data i.e phenotypic data of Nigerian F_2 upgrade chickens at 8 week; Thin black line – the expected values of Hardy Weinberg Equillibrum; Red line – corrected values).

Figure 2 Graph of comparison between Hardy Weinberg Equillibrum principle and body weight of Nigerian F_2 upgrade chicken (case i.e < 2 kg) at 8 weeks.

3.2. Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks.

All the chicken samples involved in this analysis were identified with the markers used for the measurement and computation of number of polymorphism which ranged between 45467 to 46569, the homozygosity ranged between 0.59 and 0.89, expected homozygosity which was 0.60, the variance which occurred ranged between 0.47 and 0.81, the inbreeding coefficient (F) was between 0.02 and 0.99. Generally the inbreeding coefficient was low to high. The low inbreeding coefficient was associated with the improved Nigerian chickens which are heterozygote while the high inbreeding values were for the pure Nigerian indigenous Chickens because there are more of homozygote since their gene frequencies are not yet altered. Call rate was 0.99 and the allelic heterozygosity ranged between 0.11 and 0.41 as shown on Table 1-3

A graph of Manhattan (Figure 3) was plotted to show the 42 per-id (per identity of each chicken sample) summary of Nigerian chickens samples at 8 weeks of age involved in the association test. However, this graph was plotted P-values on the y-axis against chromosomes number of the 42 Nigerian chickens sample used on the x-axis in this section of study. The green and blue bubbles indicated the SNPs on the chromosomes. This summarized the general expression of the per-ids of each chicken sample used.

Table 1 Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks

ID	No measured	No poly	Homo	EHomo	Var	F	Callpp	Het
R4	46548	46548	0.89	0.60	0.73	0.99	0.99	0.11
Z4	46378	46378	0.66	0.60	0.53	0.15	0.99	0.34
F29	46492	46492	0.78	0.60	0.75	0.47	0.99	0.21
F14	46430	46430	0.73	0.60	0.72	0.34	0.99	0.26
A31	46525	46525	0.77	0.60	0.72	0.42	0.99	0.23
K19	46496	46496	0.79	0.60	0.76	0.47	0.99	0.21
MK10	46503	46503	0.89	0.60	0.79	0.72	0.99	0.11
C17	46481	46481	0.82	0.60	0.78	0.56	0.99	0.18
A32	46434	46434	0.67	0.60	0.61	0.18	0.99	0.33
A38	46500	46500	0.77	0.60	0.64	0.42	0.99	0.23
A26	46444	46444	0.84	0.60	0.73	0.90	0.99	0.16
A18	46438	46438	0.75	0.60	0.69	0.37	0.99	0.25

ID-identity number, poly- polymorphism, Homo-homozygote, EHomo-expected homozygosity, var- variance, F-inbreeding coefficient, Callpp- call per rate, Het-heterozygote

Table 2 Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks

ID	No measured	No poly	Homo	E Homo	Var	F	callpp	Het
L31	46535	46535	0.87	0.60	0.75	0.67	0.99	0.13
L16	46437	46437	0.75	0.60	0.69	0.38	0.99	0.25
O8	46504	46504	0.76	0.60	0.69	0.40	0.99	0.24
A27	46480	46480	0.69	0.60	0.64	0.24	0.99	0.30
L4	46476	46476	0.87	0.60	0.81	0.66	0.99	0.13
L35	46466	46466	0.66	0.60	0.54	0.13	0.99	0.34
A5	46476	46476	0.76	0.60	0.67	0.40	0.99	0.23
C16	46514	46514	0.77	0.60	0.72	0.43	0.99	0.23
O11	46507	46507	0.81	0.60	0.74	0.52	0.99	0.19
F16	46526	46526	0.79	0.60	0.66	0.47	0.99	0.20
H13	46396	46396	0.59	0.60	0.47	0.40	0.99	0.41
C30	46516	46516	0.69	0.60	0.59	0.24	0.99	0.30

ID-identity number, No.Poly- polymorphism, Homo-homozygosity, EHomo-expected homozygosity, var- variance, F-inbreeding coefficient, Callpp - call per rate, Het-heterozygote.

Table 3 Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks

ID	No measured	No poly	Homo	EHomo	Var	F	Callpp	Het
C29	46486	46486	0.84	0.60	0.72	0.60	0.99	0.16
Z2	46516	46516	0.65	0.60	0.53	0.11	0.99	0.35
L12	46449	46449	0.87	0.60	0.88	0.68	0.99	0.13
R15	46547	46547	0.78	0.60	0.68	0.45	0.99	0.22
R23	46569	46569	0.68	0.60	0.59	0.21	0.99	0.32
R5	46546	46546	0.77	0.60	0.66	0.40	0.99	0.23
E4	46536	46536	0.81	0.60	0.72	0.52	0.99	0.19
F2	45467	45467	0.65	0.60	0.56	0.12	0.99	0.35
E15	46470	46470	0.71	0.60	0.54	0.02	0.99	0.29
O10	46536	46536	0.62	0.60	0.72	0.28	0.99	0.38
E18	46554	46554	0.62	0.60	0.52	0.03	0.99	0.38

ID-identity number, No.poly- Number of polymorphism, Homo-homozygosity, EHomo-expected homozygosity, var- variance, F-inbreeding coefficient Callpp- call per rate, Het-heterozygote.

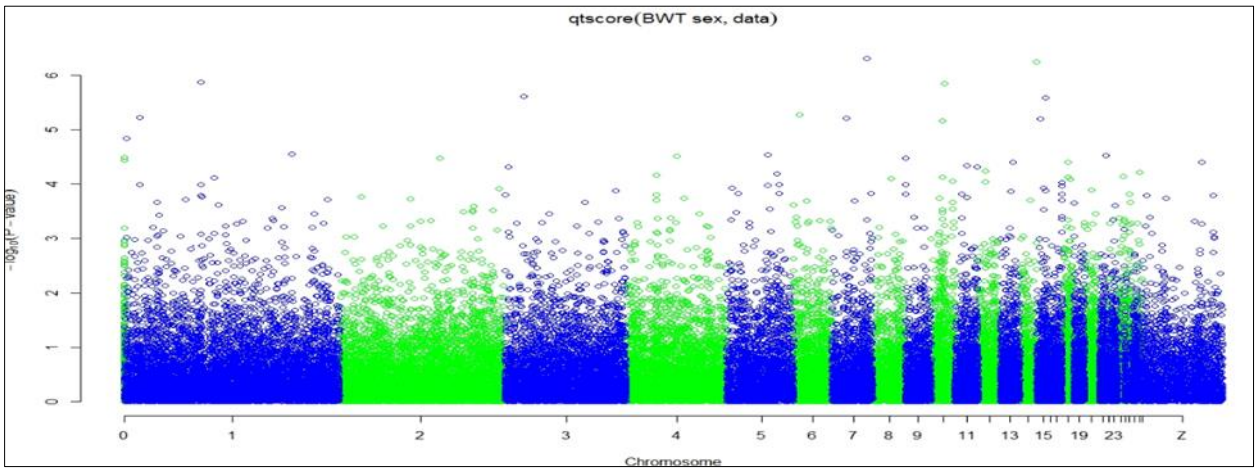


Figure 3 Manhattan plot showing Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses at 8 weeks after quality checks

3.3. Fast score test association of SNPs of Nigerian indigenous chicken and their crosses on body weights at 8 weeks.

A fast score test was conducted with the phenotypic data recorded among the 42 Nigerian chicken samples used for the association test to determine the most significant SNPs associated with body weight trait of Nigerian chickens at 8 weeks of age measured. Its location/position on the chromosome. Also, to identify alleles with homozygote or heterozygote and its substitution effects on the body weight trait and the effect of each allele and its interactive effects to this trait. The differences in the means of the effects of alleles (A1 and A2) determined the resultant effect of the individual allele B whether to be selected for improvement in respect to this study. The P-value at 1 degree of freedom tested for allelic association while at 2 degree of freedom tested for genotypic association. The principal component (Pc1df) tested for association between SNP and body weight trait and corrected the statistics for possible inflation. *An analysis of global FST showed deviations from HWE as a result of inbreeding coefficient (FIS) and in total population was low to high.* F_{IS} *was 0.02 and* F_{IT} *was 0.99.*

The most significant SNPs associated with the body weight of Nigerian improved chicken genotypes at 8 weeks of age were recorded on chromosome 14 with SNP number GGaluGA10859 at position 15592533; chromosome 7 with SNP number Gga_rs16609686 at position 33202174; chromosome 3 with SNP number Gga_rs16729741 at position 17838181; chromosome 15 with SNP number GGaluGA109681 at position 8635011; chromosome 10, SNP number Gga_rs14005805 at position 12252110; chromosome 6, SNP number Gga_rs1534214 at position 6927376, chromosome 1 with SNP number GGaluGA024649 at position 71460615; chromosome 10, SNP number Gga_rs4004904 at position 10411414, chromosome 1 number Gga_rs13831454 at position 14027733 and chromosome 7, SNP number GGaluGA312567 at position 13853781 (Table 4). A graph was plotted to present the first 10 most significantly associated SNPs with body weight on the chromosomes of Nigerian improved indigenous chicken genotypes at 8 weeks of age Figure 4.

This figure differentiates the level of significance between the body weight of Nigerian indigenous chickens and their crosses at 8 weeks of age and the SNPs on the chromosomes. The less associated chromosomes with body weight were at the P-values that ranged from 0 to 0.04 levels of significant which fell below the black demarcating line indicating 5% genome-wide significance while above it were the SNPs with P-values from 0.05 and above. These were those that were most significantly associated SNPs and chromosomes of the Nigerian improved chickens' body weight at 8 weeks that reached the genome-wide significance. However, these were the chickens with heavier body weight (Nigerian F_2 upgrade chickens) while the lighter weight chickens (pure Nigerian indigenous) were below marked P-value line.

A genome-wide association study using the chicken 60K SNP panel in Nigerian improved indigenous chicken's population derived from the cross between Marshall exotic and the pure Nigerian indigenous (Normal feathered, Frizzle feathered and Naked Neck) at 8 weeks of age was used in localizing SNPs and chromosomes region associated with the body weights of these chickens. Based on the results obtained in this study F_2 upgrade (Frizzle-Feathered and Naked Neck) were known for heavier weights which reached the 5% Bonferroni genome-wide significance threshold while the purebred Nigerian indigenous had lighter weights and could not reached the 5% Bonferroni genome-wide significance threshold. This could be due to the Marshall genetic contributions in the Nigerian chicken F_2 upgrades in which over time Marshall has been under intense selection for body weight and growth rate.

Linkage analyses provided evidence for highly ($p<0.05$) significant SNP associated with body weight of Nigerian improved indigenous genotype. Some positional SNPs on Chromosomes were identified associated with this trait of interest at different SNPs positions on chromosomes 1, 15, and 10 of the chickens.

The GGA1 had SNP significant ($p<0.05$) effects on body weight at 8 weeks of age with genome-wide significance.

The Nigerian indigenous chickens' body weight has association with the SNPS on chromosome 1 which was consistent with report by Gu *et al*. [10] who observed that chickens on chromosome 1 associated with chickens body weights using 60K SNP panel in F_2 chicken population derived from the cross between Silky Fowl and White Plymouth Rock at 7 to 12 weeks. Likewise Nassar and Brockmann [18] who confirmed that chromosome 1 of chickens has association with growth trait. SNP on chromosome 1 was found associated with body weight of Nigerian improved indigenous chickens which was in congruence with the findings of Tatsuda and Funjinaka [19] that QTL controlling chicken body weight at 7-13 weeks of age in F_2 chickens population was found on chromosome 1.

Another chromosome associated with body weight was chromosome 15 of the Nigerian improved indigenous chickens at 8 weeks of age. SNP at positional region of 112.34kb on chromosome 15 located at 8.58Mb was identified in this study. Nassar and Brockmann [18] observed that SNP on chromosome 15 significantly associated with growth traits in chickens which was in consonance with the observation of this study that chromosomes 15 associated with body weight of Nigerian improved indigenous chickens at 8 weeks of age.

The next chromosome identified associated with body weight was on chromosome 10 Nassar and Brockmann [18] observed that chromosome 10 associated with growth traits of chickens which agrees with the observation of this study that chromosomes 10 is associated with body weight of Nigerian improved indigenous chickens at 8 weeks of age.

SNPs on Chromosomes 3 and 7 were found in this study to have been significantly associated with the body weight of the Nigerian improved indigenous chickens at 8 weeks of age. The observation in this study was in consonance with the results of Ikeobi *et al*. [20] who concluded that SNPs on chromosomes 3 and 7 had a strong association with growth trait (abdominal fat) in 30 Chickens family of F_2 chicken population. However, Tatsuda and Fujinaka [19] confirmed chickens chromosome 7 to be associated with body trait (abdominal fat deposition) F_2 chicken population at 7-13 weeks of age which agrees with the result of this study that SNP on chromosome 7 significantly associated with growth trait of the

improved Nigerian indigenous chickens at 8 weeks of age and also corroborate the result by Nassar and Brockmann [18] who observed chromosome 7 to associate with growth trait.

The percentage of inbreeding coefficient was high causing a small deviation from the HWE as observed among the Nigerian chicken upgrades which is a common occurrence in commercial stocks. The genetic variation existing in chicken genotypes studied could be due to crossbreeding and selection for fast growth rate.

Table 4 Fast score test association of SNPs of Nigerian indigenous chicken and their crosses on body weight at 8 weeks

SNPS no	Chr	Position	Stra	A1	A2	N	effB	Se effB	1df	P1df	effAB	effBB	2df	p2df	Pc1df
GGaluGA105859	14	15592533	+	T	C	41	-11.92	2.23	28.64	8.73E-08	156	NA	28.64	8.73E-08	5.07E-06
Gga_rs16609686	7	33202174	+	A	G	41	-11. 37	2.68	24.84	6.24E-07	Inf	NA	24.84	6.24E-07	2.15E-05
Gga_rs16729741	3	17838181	+	A	C	40	-12.68	2.58	24.12	9.06E-07	Inf	NA	24.12	9.06E-07	2.83E-05
GGaluGA109681	15	8635011	+	A	G	42	13.78	2.84	23.49	1.26E-06	63	Inf	25.91	2.36E-06	3.61E-05
Gga_rs14005805	10	12252110	+	C	T	41	16.36	3.38	23.36	1.35E-06	52	Inf	25.04	3.66E-06	3.79E-05
Gga_rs15344214	6	6927376	+	G	A	39	-73.46	5.44	22.63	1.96E-06	Inf	Inf	23.31	8.69E-06	5.00E-05
GGaluGA024649	1	71460615	+	G	A	42	9.51	2.02	22.18	2.48E-06	26	Inf	23.15	9.41E-06	5.94E-05
Gga_rs14004904	10	10411414	+	C	T	42	6.66	1.46	20.92	4.79E-06	8	Inf	21	2.75E-05	9.66E-05
Gga_rs13831454	1	14027733	+	A	G	42	6.06	1.33	20.72	5.33E-06	4.6	Inf	21.62	2.02E-05	0.000105
GGaluGA312567	7	13853781	+	A	G	38	9.25	2.04	20.64	5.54E-06	Inf	Inf	23.27	8.87E-06	0.000108

A1 A2-Nuclotide change of Allele 1and 2, position- SNPs position, chr-chromosome number, stra-strand, effB- effect of the B allele in allelic test, se_effB-Error means of allele B, effAB- effect of heterozygosity in genotypic test, effBB-effect of homozygosity in genotypic test, pc-principal component, P1df: P-value of 1-df-degree of freedom, P2df: P-values of 2-d.f. Pc1df: P-values from the 1-d.f.

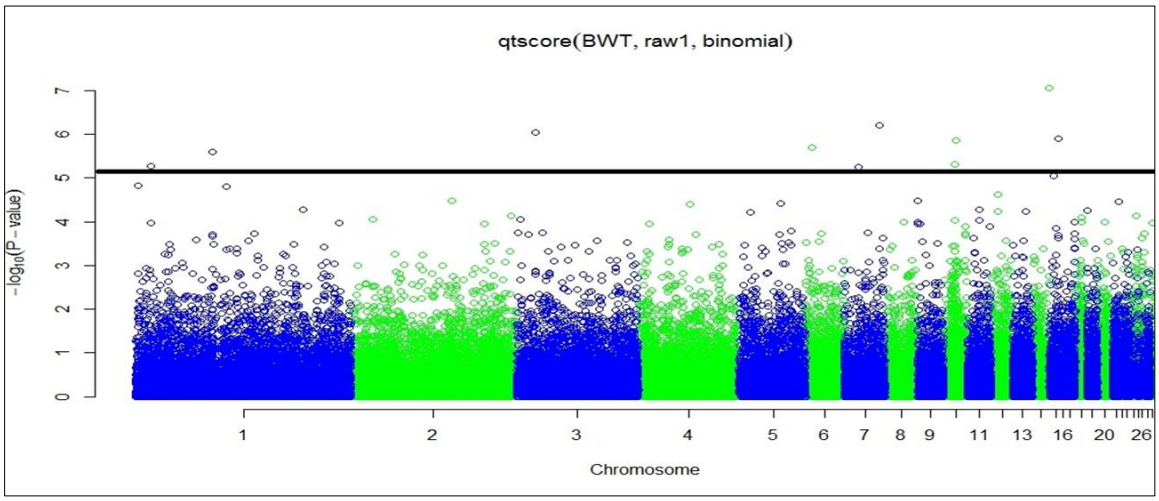


Figure 4 Manhattan plot showing the 10 most significantly associated SNPs on chromosomes with body weight of Nigerian indigenous chicken and their Crosses at 8 week.

4. Conclusion

It has been concluded: The 53313 SNPS marker used in this research passed the 5% Bonferroni Significance threshold. Genome-wide association analysis studies identified the 10 most significant chromosomes: 14, 7, 3, 15, 10, 6, 1, 10, 1, and 7 associated with body weight of Nigerian improved indigenous chicken with SNPs identified on Chromosomes 1, 15 and 10 associated with body weight in these chickens studied. There was an inbreeding coefficient of 0.02 to 0.99 indicated low to high range. In this study Illumina 60K HD Bead chip was successfully used in Nigerian improved indigenous chicken genotypes to determine SNPS and chromosomes associated with growth trait. Allele B in F_2 Frizzle upgrade chickens should be selected for body weight improvement, since it has positive effect on body weight in this study. In addition, more chromosomes should be identified if higher density chips like 600K are used

Recommendations

Introduction of exotic gene in Nigerian indigenous chicken genotype should be encouraged as this would rapidly improve the performance of Nigerian chickens. Poultry breeders should improve the Nigerian indigenous chickens to an F_2 upgrades for the achievement of excellent performance especially the Frizzle-feathered and Naked Neck for meat-type purpose. Through the practice of crossbreeding and use of marker assisted selection in the breeding program, the Nigerian chickens can be improved for commercial purposes. The sample size should be increased to reduce error while carrying out genome-wide association study with the Nigerian chicken genotypes in order to generate in-depth information that can aid selection for future use, since, this is a preliminary phase of SNPs study. Allele B in F_2 Frizzle upgrade chickens should be selected for body weight improvement, since it has positive effect on body weight in this study. These genes identified should further be studied for future selection purpose. In addition, more genes should be identified if higher density chips like 600K or more are used.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in this manuscript.

Statement of ethical approval

This research was conducted in accordance with ethical guidelines governing the use of animals and genetic materials in scientific research as stipulated by the Ethical Committee of Department of Animal Science, Faculty of Agriculture, University of Uyo, which is in line with Animal health care constitution.

Statement of informed consent

Authors have given an approval and consent for the article to be publication.

Author declaration

Authors have declared that this article is original article submitted for publication which has not been published anywhere in the world.

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